80* Transcriptional profile of human airway cells during the epithelial regeneration

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Despite the frequent injuries affecting the airway epithelium, molecular mechanisms associated with its regeneration are still poorly described. In order to determine the transcriptional profile of human airway cells during this process, airway epithelial cells were seeded on type IV collagen-coated porous membranes, cultured up to confluence in liquid-liquid (LL) conditions then at the air-liquid interface (ALI), in order to induce mucociliary differentiation. Transcriptome experiments were performed on cells at 70% confluence in LL conditions (step I), after 5 days in ALI conditions (step II), at the onset of ciliary differentiation (step III) and when cultures were completely differentiated (step IV). 127 genes were selected to discriminate step I from step II (74 up- and 53 down-regulated). Similarly, 127 transcripts were up-regulated between step II and step III (tubulin, dynein, STATH, microtubule associated proteins . . .) and only one gene (fatty acid desaturase-1) was repressed. Few genes were modulated between step III and step IV, at the notable exception of STATH (12-fold repressed). 256 genes were up-regulated between step I and step IV and 49 were repressed, including a family of genes involved in ciliary differentiation (FOXJ1, tektin, dynein, tubulin, . . .), extracellular matrix proteins (collagen, laminin, . . .) and inflammatory-related genes, growth factors and their receptors (CCL20, VEGFC, CSF3, IL32, IL13RA2, . . .).
The elucidation of time-dependent patterns of expression during the airway epithelial regeneration provides a substratum for a better understanding of this process. In the context of the regeneration of the CF airway epithelium, this work may open the way to new therapeutic avenues.
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81 Transepithelial ion transport: the relationship with long-term survivors of cystic fibrosis

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Rationale: Significant heterogeneity exists in the clinical outcome and survival rates of patients with classic CF. The aim of this study was to determine if long-term survival results from preserved CFTR functionality.
Methods: Prospective assessment of nasal potential difference (PD) and sweat chloride concentrations in 34 long-term survivors of CF (aged ⩾40 years) compared to young CF patients (aged 18–23 years) with severe (n = 30) and mild (n = 31) lung disease. All patients had 2 'severe' CFTR mutations. Differences between the 3 groups were tested by 1-way ANOVA and linear regression models were used to assess the relationship between lung function and nasal PD.
Results: Nasal PD in the long-term survivors was not statistically different to the young groups ($p>0.05$; table). Maximum stable baseline and the response to amiloride correlated with lung function (FEV_1 0.14 (95% confidence interval 0.04 to 0.24; $p = 0.009$) and –0.12 (95% CI –0.20 to –0.04; $p = 0.003$), respectively). They were lowest in the young/mild group – significantly lower than the young/severe group ($p = 0.035$ and $p = 0.001$, respectively) but not the old group ($p = 0.494$ and $p = 0.062$, respectively). Sweat chloride concentration was lowest in the young/severe group ($p < 0.05$).
Conclusion: Measurements of transepithelial sodium ion transport correlated with lung function severity, but this, and measurements of chloride transport, were not preserved in long-term survivors of CF.

Nasal PD measurements (mV) and sweat chloride concentration (mmol/L)

Group	Baseline	ΔAmiloride	ΔlowChloride	ΔlowChloride+Iso	Sweat chloride
Young/mild	–41.2 ±10.8	22.3±8.0	–0.4±3.5	–0.9±4.3	99.5±7.5
Young/severe	–47.8±12.7	29.9±9.4	–0.2±4.2	–1.0±5.8	94.0±8.9
Long-term survivors	–43.3±12.7	26.7±10.1	–0.5±4.9	–1.2±4.8	98.5±9.6

Data presented as mean±SD. Iso=isoproterenol.

82 Nasal potential difference testing – the effect of different glucose and chloride concentrations

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The nasal potential difference (PD) technique can provide useful diagnostic information and can be used to assess the physiological effect of new treatments (*Eur Respir J* 1994:7:2050–2056). However, different laboratories have developed individual protocols, with subtle differences in solutions and perfusion rates and temperature. Each of these may slightly alter the measurements of sodium and chloride transport across the airway epithelium *in vivo.* This abstract compares different glucose and chloride concentrations on the nasal PD.
Methods: Our standard diagnostic nasal PD system was used in 6 normal subjects to assess the effect of effect of 0, 10 and 20 mM glucose on the baseline nasal PD. The effect of 0 and 6 mM chloride were compared following pre-treatment with amiloride to block sodium absorption.
Results: The change from 0–10–20 mM glucose exerted little effect on nasal PD (< 2 mV). In contrast, following amiloride pre-treatment, change between 6 and 0 mM chloride increased the PD by approximately 2 mV.
Conclusion: In non-CF subjects, nasal PD protocols that use different glucose concentrations are likely to have similar values of baseline PD. However, the use of zero chloride will give responses that are approximately 2 mV greater than protocols which use low (6 mM) chloride.
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83 Lentiviral transduction of differentiated airway epithelial cells in culture is enhanced by lysophosphatidylcholine

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Efficient viral transduction of fully differentiated airway epithelium is difficult, due to effective barrier function and mucociliary clearance. Agents that disturb the integrity of the epithelium are known to improve the transduction efficiency. Here we studied the effect of lysophosphatidylcholine (LPC) on lentiviral (LV) transduction of differentiated air-liquid interface (ALI) cultures of human primary bronchial epithelial cells (PBEC). PBEC were cultured on transwell filters and allowed to differentiate in an ALI setting for 2–4 weeks. Cultures were then washed 3× with 75–300 μg/ml LPC in HBS or with HBS alone, and subsequently transduced o/n with 5×10^6 transducing units of a VSV-G pseudotyped SFFV-GFP LV vector. In addition, PBEC were transduced with the same vector before allowing differentiation on transwells.
Expression of GFP was observed in 40–50% of epithelial cells in ALI cultures treated with 75 μg/ml LPC, while 50–60% was positive at 300 μg/ml LPC. In HBS treated cultures <5% cells were GFP positive. Detailed analysis of the cultures revealed that LPC at 300 μg/ml caused shedding of cilia, while this was not the case at 75 μg/ml. No loss of cell number or tight junctions was observed after LPC treatment. Addition of LV vector prior to differentiation resulted in a transduction efficiency of 80–90% and did not appear to affect subsequent cell differentiation.
Our studies demonstrate efficient LV transduction of undifferentiated PBEC, and show considerable enhancement of LV transduction of ALI cultures. This opens opportunities for further research on gene transfer to airways using LV vectors.
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